1.0. Introduction

HIV-1 is the most important viral pathogen of humans. It is estimated that 32 million people will be living with HIV infection and that 3 million people will die during 1998 (1). Despite these sobering international statistics, morbidity and mortality related to HIV infection have been declining in the United States since 1996, largely because of improved antiretroviral therapy (2,3).

Combination antiretroviral therapy (CART) typically includes one protease inhibitor and two reverse transcriptase inhibitors, although other combinations are also used. The effectiveness of combination therapy has been demonstrated in clinical trials (4,5). The primary effect of CART is to suppress viral replication, as measured by plasma HIV-1 RNA, which decreases the rate of progression of immune deficiency (6,7,8,9).

In the future, there are several issues that will dampen the effect of CART on the epidemic. These include patients' ability to tolerate the agents, the long-term toxicity of the agents, the complicated, highly regimented treatment schedules, the development of resistance to the regimens, and the effect of previous therapy on the response to a new regimen.

The antiretroviral agents currently approved for use in the United States are generally well-tolerated; but there are significant numbers of patients who are unable to tolerate them. In clinical trials, as many as 25% of participants failed to complete the study for this reason (4). These patients require novel therapeutic interventions.

The long-term toxicity related to the use of CART is not known at the present time. It has been suggested that CART, specifically protease inhibitors, may cause disturbances in lipid metabolism (10,11). Whether this effect is related to CART therapy, or is a consequence of HIV infection only being recognized now that patients are living longer, has not been determined. Patients who develop serious long-term toxicity may need to discontinue CART and will require new therapeutic options.

The schedules for CART therapy are complicated, and patient compliance is critical for treatment success. The compliance issues are related to the number of pills that must be taken daily, the timing of these medications around meals (since food affects absorption of the various agents), and the requirement for strict adherence to the regimen in order to maintain effective drug plasma concentrations. Problems with compliance have been shown to be associated with the rapid development of resistance that may or may not be controlled when compliance with CART is resumed, with obvious consequences for the patient (12,13).

Unfortunately, CART resistance can develop even when the patient is compliant with therapy. The HIV-1 genome is single stranded, and the reverse transcriptase enzyme

is error-prone (14,15). With an error rate of one base in every few thousand copied, multiple mutations develop with each replication. The more replicative events, the higher the probability of developing resistance. These scientific observations are supported by the clinical observation that the duration of response to CART is directly related to the nadir of the plasma HIV-l RNA, which is an indirect measure of viral replication (16). Previous antiretroviral therapy also affects the response to CART. The results from many clinical trials suggest that the response to a new antiretroviral therapy, either monotherapy or CART, is related to previous therapy, particularly with similar agents (12). Previous therapy that does not control viral replication selects for viral resistance and results in a viral population at baseline that has already developed some or all of the mutations required for resistance to the new therapy. The treatment options available today have high degrees of cross-resistance within a class of agents, with the result that failure with one CART regimen diminishes the probability of having a durable therapeutic response to another regimen. Patients who develop resistance for any of these reasons require new treatment options.

New AIDS therapies can be divided into two categories: those that attempt to control viral replication and those that seek to reconstitute the immune system. Effective therapies have the potential to affect both variables. For example, there is an element of immune reconstitution that occurs with CART therapy (17). Nonetheless, each therapy primarily affects one or the other of these variables.

SyStemix has developed a novel therapeutic strategy that has immune reconstitution as its primary aim. This clinical program is based on the concept of protecting the cells that are the targets of HIV-l infection with therapeutic nucleic acid sequences that inhibit viral replication, thereby allowing survival of the target cells. Prolonged survival of $CD4^+$ T cells would be expected to lead to increased numbers of these cells, and the $CD4^+$ count has been shown to be indicative of the magnitude of immunodeficiency in persons with HIV-1 infection (18).

The therapeutic sequences used by SyStemix include the gene for the transdominant negative Rev mutant, RevM10, and an antisense sequence pol-1 to the pol gene. Rev functions to facilitate the nuclear export of unspliced RNA transcripts to the cytoplasm for translation to the viral structural proteins, gag, env and pol. In order to complete this function, two domains of the protein are essential. The first is a binding domain that binds to the Rev responsive element (RRE) on the unspliced RNA transcript. After binding, an activation domain assembles the required cofactors that make up the nuclear export complex (19). HIV-1 viruses with Rev deletions or mutations do not replicate. The RevM10 mutant has an intact binding domain that allows RevM10 to bind to RRE. It has two mutations in the activation domain that prevent assembly of the nuclear export complex, with the result that translation of structural proteins is blocked, thereby blocking viral replication (20, 21).
The pol gene yields the reverse transcriptase, integrase, and RNase H proteins. The pol-1 antisense construct used by SyStemix is complementary to the region of pol

which codes for reverse transcriptase (17). The antisense RNA binds to the complementary region of pol, forming an RNA-RNA duplex that is then degraded and is not translated to protein. This block in viral protein synthesis has been shown to result in decreased viral replication. The combination of RevM10 and polAS has been shown to inhibit viral replication to a greater extent than either sequence alone (22).

The SyStemix treatment strategy is based on the transduction of hematopoietic stem cells with vectors containing RevM10 and polAS. HSC are self-renewing so that a single treatment has the potential to result in the lifelong generation of gene-modified progeny. The cells that are the primary targets of HIV-1 infection, $CD4^+$ T cells, monocytes and macrophages, and microglial cells, are all progeny of HSC. Thus, the use of gene-modified HSC has the potential to provide a lifelong population of target cells for HIV infection that are resistant to HIV replication and HIV-induced cell death.

There are three technical issues to resolve in order to implement this technology: obtaining sufficient numbers of HSC; successful transduction of the HSC; and engraftment of the gene-modified HSC. The mobilization of HSC from persons with HIV-1 infection has been shown to be both safe and feasible. In a previous clinical trial sponsored by SyStemix, 33 persons with HIV-1 infection received 5 days of G-CSF for HSC mobilization. There was no change in viral load during the course of the study (from day 0 to day 90), and the numbers of HSC mobilized were comparable to those mobilized in patients without HIV-1 infection. These results are similar to data from similar trials in Denmark (23) and in ACTG study 285 (24).

As this therapy has developed, the transduction process used at SyStemix has been modified. The original process used vector packaged in PA317 cells with transduction efficiencies of <10% when measured by gene-marked colony-forming units (CFU). The use of a human proprietary packaging line, ProPak, and technical improvements in the transduction process, have resulted in transduction efficiencies >40%. The improved transduction process will be used in this clinical trial.

The conditions that are required for engraftment of large numbers of gene-modified HSC are not known at this time, although low level engraftment of gene-modified HSC has been demonstrated by several groups (25,26). Experience from the allogeneic bone marrow transplantation literature suggests that the combination of cyclophosphamide and total body irradiation resulted in myeloid cells that were all of donor phenotype but lymphocytes that were not all of donor phenotype. The addition of thiotepa resulted in both myeloid and lymphoid cells being completely of donor phenotype (27). In the SyStemix treatment strategy, the maximum therapeutic benefit from the gene-modified cells should be derived following a conditioning regimen that supports the greatest representation of donor (gene-modified) lymphoid cells.

The use of an intensive myelosuppressive conditioning regimen is associated with risks to the patient. These include the general and well recognized risks of autologous peripheral blood stem cell transplantation, including the toxicity of the conditioning agents and the possibility of infection during the period between conditioning and engraftment. The conditioning regimen to be used in this clinical trial has been associated with a regimen-related mortality of 9.7% at 100 days in patients undergoing allotransplantation for Acute Myelogenous Leukemia in first clinical remission (28). Those eligible for participation in this clinical trial are patients with <200 $CD4^+$ lymphocytes/mm^3 cells who have not been able to achieve control of viral replication with the use of CART (plasma HIV-1 RNA >30,000 copies/mL). These patients have a 25% chance of death in one year and 14% five-years survival (8). The potential benefit for this patient population justifies the risk of the conditioning regimen. Previous experience with myelosuppressive chemotherapy has demonstrated a transient reduction in plasma HIV-1 RNA with treatment (HK Holland, Personal Communication). This reduction in viral burden may confer a clinical benefit apart from the investigational agent.

The final safety concern in this clinical trial is related to the effect of cytokine stimulated transduction on the HSC. The ability of HSC to engraft and persist in humans is well documented. The ability of transduced HSC to engraft has been demonstrated, but the numbers of gene-marked cells have not been adequate to determine long-term persistence (25,26). In one clinical trial evaluating the transplantation of cytokine expanded $CD34^+$ selected PBPC, graft failure was seen within 30 days when differentiation of the HSC was induced during the cytokine exposure (29). The transduction protocol used by SyStemix is designed to promote division, but not differentiation, of the HSC. The ability of large numbers of transduced HSC to persist in vivo in man has not been previously demonstrated. Therefore, all patients in this clinical trial will have a backup PBPC graft collected that will be kept at the clinical site in the event that the investigational graft fails. As a safety measure, the first patient treated in this study will be evaluated as a pilot patient. If this patient fails to engraft or if the graft fails after initial engraftment, no additional patients will be enrolled in this trial. In addition to the pilot phase, if, during the course of this trial, two patients who receive RevM10polAS HSCIP fail to engraft or if their grafts fail after initial engraftment, no other patients will be enrolled, and the study will be terminated.